

Preliminary Extended Determination¹ of Nonregulated Status for Syngenta MZIR098 Corn (*Zea mays*)

In response to a request from Syngenta Seeds, Inc. (hereinafter Syngenta) to extend a determination of nonregulated status to corn event MZIR098 (15-218-01p) resistant to herbicides and insect damage, the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has determined, based on similarity to the antecedent organism, that MZIR098 corn and progeny derived from it are unlikely to pose plant pest risks and are no longer to be considered regulated articles under APHIS' Biotechnology Regulations (Title 7 of Code of Federal Regulations (CFR), part 340). This extension request is based upon APHIS' determinations of nonregulated status of an antecedent organism, Pioneer Hi-Breed International Inc. DP-ØØ4114-3 corn resistant to an herbicide and and insect damage (hereinafter Pioneer 4114 corn). Pioneer 4114 corn (petition number 11-244-01p) was deregulated on June 20, 2013. APHIS authorizations that were previously required for environmental release, interstate movement, or importation under those regulations will no longer be required for MZIR098 corn and its progeny. Importation of MZIR098 corn seeds, other propagative material, and grain for consumption would still be subject to APHIS foreign quarantine notices at 7 CFR part 319 and the Federal Seed Act regulations at 7 CFR parts 201 and 361.

MZIR098 has been engineered to express three proteins which confer resistance to herbicide and to insect damage. APHIS evaluated the plant pest risk of MZIR098 by assessing its similarity to the deregulated antecedent, Pioneer 4114 corn. This corn lines produces proteins which have the same mechanisms as do the proteins produced in MZIR098 corn.

APHIS previously conducted a Plant Pest Risk Assessment on the antecedent organism and found it unlikely to pose risks as a plant pest. Based on a the plant pest similarity assessment (see Appendix A) of MZIR098 to the antecedent, APHIS concludes that MZIR098 corn is unlikely to pose a plant pest risk and should no longer be regulated under 7 CFR part 340. From the similarity assessment APHIS concludes the following with respect to MZIR098 corn and its progeny:

- (1) No plant pest risk was identified from the transformation process, the insertion and/or expression of new genetic material, or from changes in metabolism in MZIR098 corn.
- (2) Disease and pest incidence and/or damage is not expected to be increased or atypical for MZIR098 corn. No plant pest effects are expected on these or other agricultural products and no impacts are expected to APHIS pest control programs.
- (3) Based on the proteins introduced, and their similarity to the antecedents, MZIR098 corn is unlikely to adversely impact nontarget organisms beneficial to agriculture.
- (4) MZIR098 corn is no more likely to become weedier or more difficult to control as a weed than the antecedent, which is not weedy.

¹ This extended determination is not effective until officially signed and published.

- (5) Gene introgression from MZIR098 corn into wild relatives in the United States and its territories is extremely unlikely and is not likely to increase the weediness potential of any resulting progeny nor adversely affect the genetic diversity of related plants any more than would cultivation of traditional or other specialty corn varieties.
- (6) Significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of MZIR098 corn are not expected.
- (7) Horizontal gene transfer of the new genetic material inserted into the GE plant to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

In addition to our finding that MZIR098 corn is unlikely to pose a plant pest risk, APHIS reviewed the Environmental Assessment for the antecedent and reached a Finding of No Significant Impact (FONSI) for this action. Deregulation of MZIR098 corn will have no significant impacts, individually or collectively, on the quality of the human environment and will have no effect on federally listed threatened or endangered species, species proposed for listing, or their designated or proposed critical habitats (http://www.aphis.usda.gov/biotechnology/not_reg.html).

Based on my review and consideration of all of the scientific and environmental data, analyses, information, and previous conclusions regarding the plant pest risk assessment for the antecedent organism, the plant pest risk similarity assessment, and FONSI, and my knowledge and experience as the APHIS Deputy Administrator for Biotechnology Regulatory Services, I have determined and decided that this determination of nonregulated status of MZIR098 corn is the most scientifically sound and appropriate regulatory decision.

Michael J. Firko, Ph.D.
APHIS Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

Date

Appendix A

Syngenta Seeds Inc. Request (15-218-01p) for Extension of Determination of Non-regulated Status of MZIR098 corn

OECD Unique Identifier: SYN-00098-3

Plant Pest Risk Similarity Assessment

January 2016

Agency Contact
Cindy Eck
Biotechnology Regulatory Services
4700 River Road
USDA, APHIS
Riverdale, MD 20737
Fax: (301) 734-8669

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, sex, religion, age, disability, political beliefs, sexual orientation, or marital or family status. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA'S TARGET Center at (202) 720-2600 (voice and TDD).

To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 1400 Independence Avenue, SW, Washington, DC 20250-9410 or call (202) 720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.

Mention of companies or commercial products in this report does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned. USDA neither guarantees nor warrants the standard of any product mentioned. Product names are mentioned solely to report factually on available data and to provide specific information.

This publication reports research involving pesticides. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

TABLE OF CONTENTS

A.	Introduction.....	1
B.	Development of MZIR098.....	2
C.	Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism	2
D.	Potential Plant Pest and Disease Impacts.....	5
E.	Potential Impacts on Nontarget Organisms Beneficial to Agriculture	6
F.	Potential for Enhanced Weediness of MZIR098	6
G.	Potential Impacts on the Weediness of Any Other Plants with which MZIR098 Can Interbreed.....	7
H.	Potential Changes to Agriculture or Cultivation Practices	7
I.	Potential Impacts from Transfer of Genetic Information to Organisms with which MZIR098 Cannot Interbreed.....	8
J.	Conclusion	8
K.	References.....	9
L.	Similarity Table.....	12

Introduction

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has received an extension request (15-218-01p) from Syngenta Seeds, Inc. (hereafter referred to as Syngenta). In accordance with §340.6(e)(2), Syngenta requests APHIS' determination of nonregulated status for Pioneer Hi-Breed International Inc. DP-ØØ4114-3 resistant to insect damage from coleopteran and lepidopteran species and resistant to glufosinate herbicide (hereinafter Pioneer 4114 corn) be extended to Syngenta's event MZIR098 corn which is resistant to both insect damage from coleopteran species and tolerant to the herbicide glufosinate. Nonregulated status would also be extended to crosses of MZIR098 corn with conventional corn or other nonregulated corn varieties. USDA announced its determination of nonregulated status for Pioneer 4114 corn (11-244-01p) on June 20, 2013.

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the regulatory requirements of Part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under Part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest. MZIR098 corn was produced by the *Agrobacterium tumefaciens* mediated transformation of immature embryos, and some of the introduced regulatory sequences come from plant pest organisms listed in 7 CFR 340.2 (Syngenta, 2015). Therefore, the MZIR098 corn is considered a regulated article under APHIS regulations at 7 CFR part 340.

Potential impacts in this Plant Pest Risk Similarity Assessment are those that pertain to plant pest risks associated with MZIR098 corn and its progeny and their use in the absence of confinement relative to the antecedent organism Pioneer 4114 corn. APHIS utilizes data and information submitted by the requester, in addition to current literature, to determine if MZIR098 corn is any more likely than Pioneer 4114 corn to pose a plant pest risk. APHIS specifies in 7 CFR 340.6(e) that an extension request for nonregulated status shall include information to establish the similarity of the antecedent organism to the regulated article in question. As of December 2016, APHIS has deregulated 7 GE corn varieties to resist image damage from coleopteran pests and 10 GE corn varieties to resist glufosinate herbicide. The three transgenes in MZIR098, *ecry3.1Ab*, *mcry3A*, and *pat-08*, encode proteins that are identical to those in previously deregulated corn lines 5307 (petition 10-336-01p), MIR604 corn (petition 04-362-01p), and Bt11 (petition 95-195-01p), respectively. Mechanism of action is the primary consideration for similarity with respect to plant pest risks and APHIS finds the Pioneer 4114 corn to be a suitable antecedent based on similarity. Pioneer 4114 corn is furthermore the most recent deregulation reflecting the latest literature and analysis.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology' (51 FR 23302, 1986; 57 FR 22984, 1992). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S.

Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

B. Development of MZIR098 Corn

As described in their extension request (Syngenta, 2015), Syngenta genetically engineered corn with three genes, *ecry3.1Ab*, *mcry3A* and *pat-08* to develop MZIR098 corn that provides two distinct Cry proteins to resist insect damage from coleopteran pests and to provide resistance to glufosinate derived herbicides. The native Cry3A from the soil bacterium *Bacillus thuringiensis* subsp. *tenebrionis* is active against certain coleopteran pests. The modified protein mCry3A produced by MZIR098 corn has enhanced activity against western corn rootworm (*Diabrotica virgifera virgifera*) and other related coleopteran pests of corn. The engineered protein eCry3.1Ab is a chimera of mCry3A and Cry1Ab that is also active against *D. virgifera virgifera* and other related pests of corn. The native Cry1Ab from *B. thuringiensis* subsp. *kurstaki* is active against certain lepidopteran pests; however, the portion of Cry1Ab included in eCry3.1Ab has not preserved the activity of Cry1Ab against lepidopterans. The transgene *pat-08* was derived from the soil bacterium *Streptomyces viridochromogenes*. PAT acetylates glufosinate-ammonium, thus inactivating it and conferring tolerance to glufosinate-ammonium in herbicide products, and was used as the selectable marker in development of MZIR098 corn.

Coleopteran resistance in the antecedent, Pioneer 4114 corn, utilizes Cry34/35 and Cry1F proteins rather than eCry3.1Ab and mCry3A. Cry34/35 is a binary toxin; neither protein is toxic alone. It is active against certain coleopteran pests. Cry1F is active against lepidopteran pests and can be disregarded for this similarity analysis. While the individual gut binding sites may differ among Cry proteins, eCry3.1Ab, mCry3A, and Cry34/35 share a similar general mechanism of action, namely the proteins form crystals that when consumed by insects, dissolve in the insect midgut ultimately forming pores that permeabilize the gut of coleopteran pests. Corn events with identical proteins have been previously reviewed by APHIS: eCry3.1Ab and mCry3A proteins have been reviewed previously in petitions for deregulation of corn events 5307 and MIR604, respectively. MZIR098 corn expresses phosphinothricin acetyltransferase (PAT) protein which has the same mechanism of action though not the identical sequence to the PAT protein expressed in Pioneer 4114 corn. In addition, it is identical to the PAT protein expressed in the corn line Bt11, which was previously reviewed by APHIS.

APHIS BRS completed an environmental assessment (EA) and a plant pest risk assessment for Pioneer 4114 corn (USDA, 2013b and USDA, 2013c). The EA fully addressed all environmental impact issues of potential concern. In the petition, APHIS concluded on the basis of the EA that the impacts would not be significant. The agency issued a Finding of No Significant Impacts (FONSI) and made a determinations of nonregulated status. The Cry proteins and PAT proteins have a history of safe use in agricultural crop commodities.

C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism

To inform APHIS of the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNA and its expression products, APHIS assessed data and information presented in the extension request related to the similarity of MZIR098 corn to Pioneer 4114 corn: the transformation process; the source of the inserted genetic material and its function in

both the donor organism and the GE crop event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction and the number of loci inserted.

APHIS also assessed data presented in the extension request on whether the genetic modification results in expression of new genes, proteins, or enzymes or changes in plant metabolism or composition in the MZIR098 corn. The assessment encompasses a consideration of the expressed Cry and PAT proteins and any observed or anticipated effects on plant metabolism including, e.g. any relevant changes in levels of metabolites, antinutrients, or nutrients in forage and grain derived from the MZIR098 corn compared to Pioneer 4114 corn or unmodified corn.

Description of the genetic modification and inheritance of inserted DNA

Syngenta used the transformation plasmid pSYN17629 to produce MZIR098 corn through *Agrobacterium tumefaciens* mediated transformation as described in Syngenta's extension request (Syngenta, 2015, p. 22). Syngenta engineered the pSYN17629 plasmid to contain expression cassettes for *ecry3.1Ab*, *mcry3A* and *pat-08* which confer dual resistance to coleopteran pests and glufosinate resistance respectively.

Expression cassette *ecry3.1Ab*:

- NOS-02 Enhancer: sequence from the NOS gene of *A. tumefaciens* which increases gene expression (NCBI accession number V00087.1) (Bevan et al., 1983)
- CMP-04 promoter: Cestrum yellow leaf curling virus promoter region (Hohn et al., 2007). Provides constitutive expression in corn.
- *ecry3.1Ab*: An engineered Cry gene active against certain corn rootworm (*Diabrotica*) species (NCBI accession number GU327680.1). As an engineered chimeric protein, eCry3.1Ab has similarities to other well-characterized Cry proteins. Because some Cry proteins share structural similarities, chimeric Cry genes can be engineered via the exchange of domains that are homologous between different Cry genes. The gene *ecry3.1Ab* consists of a fusion between the 5' end (domain I, domain II, and 15 amino acids of domain III) of a modified Cry3A gene (*mcry3A*) and the 3' end (domain III and variable region 6 [Höfte and Whiteley 1989]) of a synthetic Cry1Ab gene (see description of *mcry3A* and *cry1Ab* below). Upstream of the *mcry3A* domain, the gene *ecry3.1Ab* carries a 67-bp oligomer extension at its 5' end, which was introduced during the engineering of the variable regions and is translated into the following 22 amino acid residues: MTSNGRQCAGIRPYDGRQQHRG. The next 459 amino acid residues are identical to those of *mCry3A*, followed by 172 amino acid residues of Cry1Ab. Description of *cry1Ab*: The gene *cry1Ab* was originally cloned from *B. thuringiensis* subsp. *kurstaki* strain HD-1 (Geiser et al. 1986). Its amino acid sequence has been codon-optimized (Koziel et al. 1997) to accommodate the preferred codon usage for corn (Murray et al. 1989).
- NOS-05-01: Terminator sequence from the NOS gene of *A. tumefaciens* (NCBI, 2014, accession number V00087.1).

Expression cassette *mcry3A*

- Ubi1-18 promoter: Promoter region from *Z. mays* polyubiquitin gene which contains the first intron (NCBI, 2014, accession number S94464.1), in which three basepairs have been altered to remove restriction sites. It provides constitutive expression in monocots (Christensen et al. 1992).
- *mcry3A*: A corn-optimized *cry3A* was synthesized to accommodate the preferred codon usage for corn (Murray et al. 1989). The synthetic sequence was based on the native Cry3A protein

sequence from *B. thuringiensis* subsp. *tenebrionis* (Sekar et al. 1987). The corn-optimized gene was then modified to incorporate a consensus cathepsin G protease recognition site within the expressed protein. The amino acid sequence of the encoded mCry3A corresponds to that of the native Cry3A, except that (1) its N-terminus corresponds to M-48 of the native protein and (2) a cathepsin G protease recognition site has been introduced, beginning at amino acid residue 155 of the native protein. This cathepsin G recognition site has the amino acid sequence AAPF, and has replaced the amino acids V-155, S 156, and S-157 in the native protein (Chen and Stacy 2007).

- NOS-20 terminator: Terminator sequence from the NOS gene of *A. tumefaciens* (NCBI, 2014 accession number V00087.1). Variation of the NOS-05-01 terminator with nucleotide changes to eliminate cross-border unintended open reading frames (ORFs). Provides a polyadenylation site (Bevan et al. 1983).

Expression cassette *pat-08*:

- 35S-04 promoter region of CMV (Odell *et al.*, 1985).
- *pat-08* gene from *S. viridochromogenes* strain Tü494; confers resistance to phosphinothricin. The native coding sequence (Wohlleben *et al.* 1988) was codon-optimized for enhanced expression (NCBI, 2014, accession number DQ156557.1).
- NOS-05-01 terminator from *Agrobacterium tumefaciens*. (NCBI, 2014, accession number V00087.1)

In addition to the above genetic elements, the inserted T-DNA contains short non-coding intervening DNA sequences. These intervening sequences contain restriction enzyme recognition sites used for cloning purposes. The T-DNA also contains the left and right border sequences (approximately 25 bps each) from the *Agrobacterium tumefaciens* Ti plasmid.

APHIS reviewed the information provided by Syngenta in the extension request and determined the following:

- The T-DNA inserted into the corn genome is present at a single locus and contains a single copy of the transgene.
- The T-DNA is stably inherited from generation to generation.
- MZIR098 corn does not contain any back bone sequence of extraneous DNA fragments from the transformation plasmid pSYN17629.
- During the transformation process, 21 base pairs of the left border sequence and 22 base pairs of the right border sequence of the T-DNA were truncated. These sequences are outside of the functional DNA elements and are not expected to impact expression of the transgenes.

History of Safe Exposure

As described in Syngenta's extension request (Syngenta, 2015), the safety of eCry3.1Ab in existing commercial transgenic crop products is supported by a permanent exemption from food and feed tolerances in corn in the U.S. (EPA, 2012a). Insecticidal Cry proteins from *B. thuringiensis* have a long history of safe use in food crops. Their modes of action are highly specific within narrow ranges of related insect species and are not relevant to mammals or other vertebrates (EPA, 2012b). The eCry3.1Ab protein produced in MZIR098 corn is identical to the eCry3.1Ab protein produced in 5307 corn (OECD Unique Identifier SYN-Ø53Ø7-1) as determined by nucleotide sequencing. Event 5307 corn was the subject of an FDA consultation, as summarized in Biotechnology Consultation Note to File BNF No. 000128, dated February 29, 2012

The safety of mCry3A in existing commercial transgenic crop products is supported by a permanent exemption from food and feed tolerances in corn in the U.S. (EPA, 2007a). The mCry3A protein produced in MZIR098 corn is identical to the mCry3A protein produced in MIR604 corn (OECD Unique Identifier SYN-IR604-5) as determined by nucleotide sequencing. MIR604 corn was the subject of an FDA consultation, as summarized in Biotechnology Consultation Note to File BNF No. 000099, dated January 30, 2007.

The safety of the PAT proteins has been previously established (Herouet, 2005) (ILSI, 2011) (OECD, 1999a; OECD, 1999b). The safety of PAT in existing commercial transgenic crop products is supported by a permanent exemption from food and feed tolerances in all crops in the U.S. (EPA, 2007b). The PAT protein expressed in MZIR098 corn has the identical amino acid sequence, as determined by nucleotide sequencing, as the PAT protein expressed in a previously deregulated, commercially available GE corn, Bt11 corn (USDA, 1996).

Expression of inserted DNA and changes in gene expression, new proteins or metabolism

mCry3.1Ab was expressed in leaves, roots, and kernels but was virtually absent from pollen. mCry3A was expressed in leaves, roots, kernels, and pollen. PAT was expressed at a relatively low level in leaves and roots. It was not measurable in pollen or kernels. None of the three transgenic proteins were detected as fusions to form new proteins. Compositional analyses indicate that the levels of the majority of nutritional components did not differ between MZIR098 and near-isogenic, nontransgenic control corn, and that those levels that did differ fell within ranges considered to be normal for conventional corn (Syngenta, 2015).

D. Potential Plant Pest and Disease Impacts

APHIS assessed data and information presented in the extension request related to the similarity of MZIR098 corn to Pioneer 4114 corn to determine whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences from plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in MZIR098 corn that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses. APHIS also assessed whether MZIR098 corn is more likely to have significantly increased disease and pest susceptibility as compared to Pioneer 4114 corn. Impacts or changes in similarity to the antecedent were assessed to determine if they would (1) affect MZIR098 corn and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2) result in the introduction, spread, and/or creation of a new disease; and/or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States; and supports trade and exports of U.S. agricultural products. PPQ responds to new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be an emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs exist, however, none specifically target pests of MZIR098 corn.

Corn itself is not considered a plant pest in the United States (7 CFR 340.2). MZIR098 corn eCry3.1Ab protein has an identical amino acid sequence as the corn eCry3.1Ab protein produced in 5307 corn (USDA, 2013a). Similarly, MZIR098 corn mCry3A protein has an identical amino acid sequence as the MIR604 corn (USDA, 2007). The impact of this Cry protein on coleopterans is similar to the Cry 34/35 proteins found in Pioneer 4114 corn. Additionally, MZIR098 expresses the protein phosphinothricin acetyltransferase (PAT) which is identical to the PAT protein expressed in Bt11 (USDA, 1996) and functionally equivalent to the PAT protein expressed in Pioneer 4114 corn.

Because the eCry3.1Ab and mCry3A proteins in MZIR098 are similar in function to the Cry34/35 protein from the antecedent Pioneer 4114 corn, and identical to those in previously deregulated 5307 and MIR604 corn, respectively, , and the PAT protein is functionally equivalent to the PAT protein in the antecedent Pioneer 4114 corn and identical to the PAT protein in Bt11 , and these events were found to not have significant changes in composition, no significant changes in composition are expected from the expression of these genes in MZIR098 and none were observed for the majority of nutrients analyzed. Similarly, MZIR098 is not expected to differ from the antecedent in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

APHIS has previously evaluated the potential impacts on nontarget organisms beneficial to agriculture that could result from the deregulation of Pioneer 4114 corn expressing the Cry34/35 protein from *Bacillus thuringiensis* and the PAT protein from *Streptomyces viridochromogenes*. This antecedent was found to be unlikely to have an adverse effect on nontarget organisms in the environment. The eCry3.1Ab and mCry3A proteins expressed in MZIR098 corn function in a manner similar to the Cry34/35 proteins expressed in Pioneer 4114. The Cry proteins from MZIR098 and Pioneer 4114 have specificity for coleopteran insects and form crystalline inclusions that once ingested by insects are solubilized in the midgut, the toxins are proteolytically activated by midgut proteases, bind to specific receptors on the surface of the midgut epithelial cells, and form non-selective pores that permeabilize the cells leading to insect death (Palma, 2014).

APHIS has also previously evaluated the potential impacts on non-target organisms beneficial to agriculture that could result from the deregulation of Syngenta events 5307 and MIR604 which express the identical eCry3.1Ab and mCry3A proteins, respectively as found in MZIR098. Both of these corn events were found to be unlikely to have an adverse effect on nontarget organisms in the environment.

In addition, the PAT protein expressed in MZIR098 corn is functionally equivalent to the PAT protein expressed in the antecedent, Pioneer 4114 corn as well as other GE crops with de-regulations dating back to 1995 (USDA, 1995). It is identical to the PAT protein found in corn event Bt 11. The PAT protein also has a long history of safe use.

Based on the high similarity of MZIR098 corn to the antecedent expressing similar proteins, and on the finding that the antecedent organism was unlikely to harm nontarget organisms, APHIS concludes that it is unlikely that MZIR098 corn will have an adverse effect on nontarget organisms, including those beneficial to agriculture.

F. Potential for Enhanced Weediness of MZIR098 Corn

APHIS has previously assessed the potential of the antecedent corn event, Pioneer 4114 corn, to become a weed. In addition, APHIS has also assessed the potential weediness of many other genetically engineered corn events representing a variety of traits. For both the antecedent and other GE corn, it was concluded that the new traits would not make the corn any more likely to become a weed. And therefore, because of the similarity of MZIR098 to this antecedent, MZIR098 is no more likely to be a weed either.

The biology of corn is well studied and understood. As documented in the PPRAs of the antecedent organism, corn is not listed as a weed (Crockett, 1977; Muenscher, 1980), nor is it present on the Federal Noxious Weed List (7 CFR part 360.200). Corn possesses few of the characteristics of those plants that are notably successful as weeds (Baker, 1974; Keeler, 1989).

In addition to considerations of the known biology of corn, APHIS analyzed information submitted in the petitions on the antecedent organism on a suite of agronomic phenotypic characteristics and plant-disease and plant-insect interactions. This agronomic data from the field showed that the antecedent was not different than its non-transgenic comparator. The assessments concluded that the antecedent was unlikely to become a weed. Based on the high similarity of MZIR098 corn to the antecedent expressing similar proteins, and on the finding that the antecedent organism was unlikely to become a weed, APHIS concludes that it is unlikely that MZIR098 corn will become a weed.

G. Potential Impacts on the Weediness of Any Other Plants with which MZIR098 Corn Can Interbreed

APHIS evaluated the potential for gene introgression to occur from the antecedent corn event, Pioneer 4114 corn, to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Those assessments found that while first generation hybrids can be formed with corn's closest relative, teosinte, the hybrids are weak and do not contribute to gene flow in subsequent generations. Also, the geographic distribution of teosinte is highly limited in the United States to fairly rare, sparsely dispersed feral populations in Florida. *Tripsacum* is not as closely related to corn as teosinte, but can be successfully hand crossed with corn to form hybrids. However, the many biological and geographic constraints such as distribution, genetic incompatibility, and temporal separation of flowering time make gene flow nearly impossible. Thus introgression from cultivated corn to either of these wild relatives is highly unlikely.

These sexually compatible relatives of corn are not considered to be weeds in the United States (Holm *et al.*, 1979) and the PPRAs of the antecedents conclude that in the highly unlikely event that they acquire the new traits through gene flow; the traits would not be expected to transform them into weeds. Based on the high similarity of MZIR098 corn to the antecedent expressing similar proteins, and on the finding that the antecedent organisms were unlikely to cause wild relatives to become weeds, APHIS concludes that it is unlikely that MZIR098 corn will cause the wild relatives of corn to become weeds.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from the antecedent corn event, Pioneer 4114 corn is likely to impact plant diseases or pests or their

management, including any APHIS control programs. This includes consideration of any changes in pesticide applications, tillage, irrigation, harvesting, etc. as they relate to plant pests and diseases.

APHIS did not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, rotations, management of volunteers, etc.) from the antecedent corn event, Pioneer 4114 corn and concluded that, no impact on plant diseases or pests or their management is likely to occur. Based on the similarity of MZIR098 to the antecedent expressing similar proteins, APHIS concludes that it is unlikely that any significant changes to agriculture or cultivation practices would be associated with MZIR098 corn and therefore no impact on plant diseases or pests of their management is likely to occur.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which MZIR098 Cannot Interbreed

APHIS has previously examined the potential for the antecedent corn event Pioneer 4114 corn expressing Cry34/35 and the PAT protein to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. APHIS has similarly examined the potential for the corn events 5307 and MIR604 expressing eCry3.1Ab and mCry3A, respectively, to be horizontally transferred without sexual reproduction to other organisms. The horizontal gene transfer between unrelated organisms is one of the most intensively studied fields in the biosciences since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge *et al.*, 1998). Potential risks from stable horizontal gene transfer (HGT) from GE organisms to another organism without reproduction or human intervention were recently reviewed (Keese, 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. APHIS as reviewed the potential for horizontal gene transfer for the antecedent in numerous GE crops and and concluded that the likelihood was extremely low likelihood (USDA, 2016)

APHIS previously concluded that HGT of the inserted genetic material from the antecedent corn event Pioneer 4114 corn expressing Cry34/35 and PAT protein to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. Therefore, APHIS concludes that HGT from MZIR098 corn to other organisms is also highly unlikely.

J. Conclusion

APHIS has reviewed the information submitted in the extension request, supporting documents, and other relevant information to assess the similarity of plant pest risk of the MZIR098 corn compared to corn event Pioneer 4114 corn expressing Cry34/35 and PAT protein. APHIS concludes that the MZIR098 corn is **no more likely** to pose a plant pest risk than the previously deregulated antecedent event Pioneer 4114 corn.

K. References

- Baker, H (1965) "Characteristics and modes of origin of weeds." *The genetics of colonizing species*. Ed. Baker, HG and GL Stebbins. Academic Press. p 147–72.
- Bevan M, Barnes WM, Chilton M-D. (1983). Structure and transcription of the nopaline synthase gene region of T-DNA. *Nucleic Acids Research* 11:369–385.
- Chen E, Stacy C. (2007). Modified Cry3A toxins. Syngenta Participations AG, assignee. U.S. Patent No. 7,276,583. Washington, DC: U.S. Patent Office.
- Christensen AH, Sharrock RA, Quail PH. (1992) "Corn polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation". *Plant Molecular Biology* 18:675–689.
- Crockett, L (1977) *Wildly Successful Plants: North American Weeds*. University of Hawaii Press.
- Dröge, M; Pühler, A; and Selbitschka, W (1998) "Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern." *Journal of Biotechnology*. 64 (1): p 75–90.
<http://www.sciencedirect.com/science/article/pii/S0168165698001059> >.
- Geiser M, Schweizer S, Grimm C. (1986). The hypervariable region in the genes coding for entomopathogenic crystal proteins of *Bacillus thuringiensis*: nucleotide sequence of the kurhd1 gene of subsp. kurstaki HD-1. *Gene* 48:109–118.
- Herouet, C., Holsapple, M., Ladies, G.S., Landry, T.D., MacIntosh, S.C., Rice, E.A., Privalle, L.S., Steiner H.Y., Teshima, R., van Ree, R., Woolhiser, M., and Zawodny, J. (2004) "A multilaboratory evaluation of a common in vitro pepsin digestion assay protocol used in assessing the safety of novel proteins". *Regulatory Tox. Pharm.* 39, 87-98.
- Höfte H, Whiteley HR. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological Reviews* 53:242–255.
- Hohn T, Stavolone L, De Haan P, Ligon H, Kononova M. (2007). Cestrum yellow leaf curling virus promoters. Syngenta Participations Ag, assignee. U.S. Patent No. 7,166,770. Washington DC: U.S. Patent Office.
- Holm, LG; Pancho, JV; Herberger, JP; and Plucknett, DL (1979) *A geographical atlas of world weeds*. Krieger Publishing Company.
- ILSI. (2011). *A Review of the Environmental Safety of the PAT Protein*. Washington DC: Center for Environmental Risk Assessment, International Life Sciences Institute Research Foundation.
http://cera-gmc.org/docs/cera_publications/pub_05_2011.pdf
- Keeler, KH (1989) "Can genetically engineered crops become weeds?" *Bio/Technology*. 7 p 1134-39.

- Keese, P (2008) "Risks from GMOs due to horizontal gene transfer." *Environmental Biosafety Research*. 7 (3): p 123-49. <http://www.ebr-journal.org/action/displayAbstract?fromPage=online&aid=8208895> .
- Koziel MG, Desai NM, Lewis KS, Kramer VC, Warren GW, Evola SV, Crossland LD, Wright MS, Merlin EJ, Launis KL, Rothstein SJ, Bowman CG, Dawson JL, Dunder EM, Pace GM, Suttie JL. (1997). Synthetic DNA sequence having enhanced insecticidal activity in corn. Ciba-Geigy, assignee. U.S. Patent No. 5,625,136. Washington, DC: U.S. Patent Office.
- Muenschler, WC (1980) *Weeds. Second Edition.*: Cornell University Press.
- Murray EE, Lotzer J, Eberle M. (1989). Codon usage in plant genes. *Nucleic Acids Research* 17:477–498.
- NCBI. (2014). NCBI Protein Database. Bethesda, MD: National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health. <http://www.ncbi.nlm.nih.gov/sites/entrez?db=protein> (updated December 1, 2014).
- Odell JT, Nagy F, Chua NH. (1985) Identification of DNA sequences required for the activity of the cauliflower mosaic virus 35S promoter. *Nature* 313:810–812.
- OECD (1999a). Consensus document on general information concerning the genes and their enzymes that confer tolerance to glyphosate herbicide. Paris, France: Environment Directorate, Organisation for Economic Co-operation and Development. 26 pp. <http://www.oecd.org/env/ehs/biotrack/46815618.pdf>
- OECD (1999b). Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide. Paris, France: Environment Directorate, Organisation for Economic Co-operation and Development. 26pp. <http://www.oecd.org/env/ehs/biotrack/46815628.pdf>
- Palma, L., Monuz, D, Berry, C, Murillo, J, and Caballero, P. (2014). *Bacillus thuringiensis* toxins: An overview of their biocidal activity. *Toxins* 6 (12).
- Sekar V, Thompson DV, Maroney MJ, Bookland RG, Adang MJ. 1987. Molecular cloning and characterization of the insecticidal crystal protein gene of *Bacillus thuringiensis* var. *tenebrionis*. *Proceedings of the National Academy of Sciences of the United States of America* 84:7036–7040.
- Syngenta (2015) Request for an extension of determination of nonregulated status for insect-resistant and herbicide-tolerant MZIR098 corn.
- USDA (1995) Final EA Combined Documents 94-357-01p. https://www.aphis.usda.gov/brs/aphisdocs2/94_35701p_com.pdf
- USDA (1996) Final EA Combined Documents 95-195-1p. http://www.aphis.usda.gov/brs/aphisdocs2/95_19501p_com.pdf

- USDA (2007) Final Combined Documents 04-362-01p.
https://www.aphis.usda.gov/brs/aphisdocs2/04_36201p_com.pdf
- USDA (2013a) Final PPRA 10-336-01p.
https://www.aphis.usda.gov/brs/aphisdocs/10_33601p_dpra.pdf
- USDA (2013b) Final EA 11-244-01p.
https://www.aphis.usda.gov/brs/aphisdocs/11_24401p_fea.pdf
- USDA (2013c) Final PPRA 11-244-01p.
http://www.aphis.usda.gov/brs/aphisdocs/11_24401p_fprra.pdf
- USDA (2016) Petitions for nonregulated status (table with links to decision documents).
https://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml
- U.S. EPA. (2007a). *Bacillus thuringiensis* modified Cry3A protein (mCry3A) in corn; exemption from the requirement of a tolerance. 40 CFR §174.532.
- U.S. EPA. (2007b). Phosphinothricin acetyltransferase (PAT); exemption from the requirement of a tolerance. 40 CFR 174.522.
- U.S. EPA. (2012a). *Bacillus thuringiensis* eCry3.1Ab protein in corn; exemption from the requirement of a tolerance. 40 CFR §174.532.
- U.S. EPA. (2012b). *Biopesticides Registration Action Document: Bacillus thuringiensis eCry3.1Ab insecticidal protein and the genetic material necessary for its production (via elements of vector pSYN12274) in 5307 Corn (SYN-Ø53Ø7-1)*.
<http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2012-0108-0010>
- Wohlleben W, Arnold W, Broer I, Hillemann D, Strauch E, Pühler A. 1988. Nucleotide sequence of the phosphinothricin *N*-acetyltransferase gene from *Streptomyces viridochromogenes* Tü494 and its expression in *Nicotiana tabacum*. *Gene* 70:25–37.

Appendix B - Similarity Table

Extension Request and Petitions of Antecedents		Extension Request MZIR098 Petition 15-218-01p	Antecedent Pioneer 4114 Petition 11-244-01p	Comments	
Organism		corn	corn		
Phenotype		Coleopteran Insect and glufosinate resistance	Coleopteran, Lepidopteran Insect and glufosinate resistance	10 previous deregulations with glufosinate phenotypes 7 previous deregulations with coleopteran insect resistance.	
Genotype	Cry	<i>ecry3.1Ab</i> from <i>B.thuringiensis</i> (fusion of <i>cry3A</i> and <i>cry1Ab</i> <i>nos</i> enhancer from <i>A. tumefaciens/Cestrum yellow leaf curling virus promoter/nos terminator</i>	<i>cry34</i>	Different types of Cry proteins with similar function. Cry34/35 op binary toxin.	
			Maize ubiquitin promoter and potato proteinase inhibitor II terminator	Constitutive expression in both cases	
	Cry		<i>mcry3A</i>	ecCry3.1Ab + mCry3A may provide better resistance management with cry34/35.	
			maize ubiquitin promoter and <i>nos</i> terminator	wheat peroxidase promoter and potato proteinase inhibitor II terminator	Constitutive expression in both cases
			pat	<i>pat</i> from <i>Streptomyces viridochromogenes</i>	<i>pat</i> from <i>Streptomyces viridochromogenes</i>
	Promoter from 35S <i>CaMV</i> and <i>nos</i> terminator from <i>Agrobacterium</i>	Same promoters			
Transformation Method		<i>Agrobacterium tumefaciens</i> –mediated	<i>Agrobacterium tumefaciens</i> –mediated	Same	
Insert and Copy Number		Single intact insertion	Single intact insertion	Same	
Backbone Absent		Yes	Yes	Same	
Compositional analysis		Compositionally equivalent to conventional corn	Compositionally equivalent to conventional corn	Same	

Mechanism of Action	eCry3.1Ab	Cry34/35	Similar: MZIR098 has two re and modified Cry proteins tha confer resistance to coleopter eCry3.1Ab is a fusion protein Cry3 and Cr1Ab, and mCry3. modified form of Cry3A. Th proteins have slightly differer binding site specificity. Pione expresses Cry34 and Cry35. T proteins are only active as a p They share little sequence sin to eCry3.1Ab and mCry3A b function in a similar manner t permeabilize coleopteran mid cells.
Mechanism of Action	mCry3A		
Mechanism of Action	PAT	PAT	Same-
Date of antecedent EA/ EIS		June 2013	
Disease and pest susceptibilities	Similar as antecedents	Unlikely to change disease and pest susceptibilities	
Impacts on beneficial non-targets	Similar as antecedents	Unlikely to impact beneficial non-target organisms	
Enhanced weediness	Similar as antecedents	Unlikely to enhance weediness	
Enhanced weediness of relatives	Similar as antecedents	Unlikely to enhance weediness of relatives	
Changes to agriculture or cultivation practices	Similar as antecedents	Unlikely to change agriculture or cultivation practices	
Horizontal Gene Transfer	Similar as antecedents	Unlikely to affect the probability of horizontal gene transfer	
Plant Pest Risk	Similar as antecedents	Unlikely to pose a plant pest risk	